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THE OVER-WINTERING OF BACILLUS AMYLOVORUS

by

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THE OVER-WINTERING OF BACILLUS AMYLOVORUS

I. INTRODUCTION

Bacillus amylovorus (Burrill) Trev. is the bacterial parasite which causes fire blight. During recent years, this malady had done great damage to the apple industry of Wisconsin, it being one of the worst diseases affecting the apple in this state. Young orchards and nursery stock suffer most severely from the trouble. Probably no disease of plants has been more written about in America than the pear blight. However, only a slight amount of work has been done in relation to the over-wintering of the causal organism and the writer has undertaken to carry on an investigation, under Wisconsin conditions, of this particular part of the life history of Bacillus amylovorus. Preliminary to discussing the results of these original studies a short review of the disease will be given as worked out by the older investigators.

II. HISTORY OF THE DISEASE

1. Names. The disease has been known under the following various names: blight, fire blight, pear blight, blossom blight, twig blight, fruit blight, body blight, and canker. Sun scald injury was often confused with fire-blight and this term has been used to designate the injury done by Bacillus amylovorus.

2. Occurrence. The disease undoubtedly occurred natively on such wild pomaceous fruits as the wild crab-apple and hawthorn of eastern North America. From this source it spread to our cultivated varieties upon which it was first observed and described, about 1780, by William Denning (1794), an orchardist of the Hudson River Highlands in New York State. Fire blight gradually spread westward with the fruit industry, and reached the Pacific Coast in

California and British Columbia between 1895 and 1897. The fire blight now occurs in practically every section of the United States and Canada where pomaceous fruits are grown. As yet no authentic records have been made of its occurrence in Europe or any of the other continents. In 1881, Geo. P. Peffer (1882) of Pewaukee, Wisconsin reported the disease in his orchards. During this year he made a special study of apple blossoms and reported that the first blossoms appeared June fourth and fifth. "The next few days were hot with dry southwest winds, the thermometer standing at 90 to 96 degrees. The petals soon turned brown followed by the blackening of the leaves just below the flowers and the exudation of the "black liquid usually seen with fire blight". Apples and crabs were affected alike on both the north and south sides of the trees. "All the trees that were in full bloom during those hot days were thus blighted, and in a few days looked as though a fire had run through them, while the same varieties standing only a few rods apart that had no blossom stems or were not yet in bloom, escaped altogether, not only then, but all through the season". The author states further that the blight starting in the flower passed down the stem to the shoot and then infected the entire cluster of blossoms. From here it worked down into the new wood and leaves. Specimens of these blighted blossoms and twigs were sent Professor Burrill, who replied under date of January 19, 1882, that "there is no question but that your idea that the blight starts in the blossoms in these cases is true. It is quite new to me, but I now find similar evidences of the fact here. Professor Burrill's theory of the bacterial cause of blight, which will be discussed under another heading, was not accepted by Mr. Peffer. Burrill's experiments failed to

convey the disease without puncturing the shoot which led Peffer to believe that since the bacteria cannot gain entrance thru the uninjured parts, they could not of themselves start the disease. He believed that the cause of blight was a deficiency of sap during these hot days which wilted the flowers, or that it was a freezing of the sap which injured the cellular structure, so that when warm weather set in fermentation began. His remedies for the disease were to cut out affected parts and to apply lime and sulphur to the tree. If the weather gave indications of blight, the trees were mulched, or salt was applied to keep down the temperature.

3. Theories as to the cause. Previous to this time and before the true nature of the disease was known, many other theories as to the cause of fire blight were advanced. W. G. Sackett (1905) summarized these as follows:

1. Insects.
2. Rays of the sun passing thru vapors.
3. Poor soil.
4. Violent changes in the temperature of the air or the moisture of the soil.
5. Sudden change from sod to high tillage resulting in surplus of sap.
6. Effect of age.
7. Autumn freezing of unripe wood, which makes a poison destroying the shoots and branches the following spring.
8. Electricity in the atmosphere.
9. Freezing of the sap or freezing of the bark.
10. Fermentation of sap.

11. Absence of certain mineral matters in the soil.

12. Something in the air which is carried from place to place.

13. Fungi.

The specific cause of the disease was not suggested until 1878, nearly a century after blight had been known. Professor Burrill (1879) established, by thorough researches, the presence of bacteria in the tissues of dying limbs and the scarred blotches upon the trunks of apple and pear trees. He states that "if we remove the bark of a newly-affected limb, and place a little of the muscilaginous fluid from the browned tissues under our microscope, the field is seen to be alive with moving atoms known in a general way as bacteria. Sometimes a thick, brownish fluid oozes from the bark of dying limbs and spreads over the outside or falls in drops. This is apparently made up of the living things, myriads of them to be seen at once. A particle of this viscous fluid introduced upon the point of a knife into the bark of a healthy tree is in many cases followed by blight of the part, but with me not in every instance". Examination of these inoculated trees showed the presence of bacteria in advance of the discolored portions of the tissues, which led Burrill to distinctly state that he believed the malady to be due to bacteria. July 1, 1880, a series of experiments was started by him to determine whether these organisms were really active in the observed changes, or simply accompanied other agents of destruction. One year later (1881), he published the results of his inoculation experiments whereby he produced the disease in healthy pear and quince trees by inoculation with virus obtained from diseased pear. Inoculation of pear with bacterial material from

blighted apple produced a thorough infection which led Burrill to believe that the disease is identical in the two trees. His experiments on apple trees failed to produce the disease by inoculation with pear or apple virus. Burrill's inoculations in his first experiments were made by inserting cuttings of diseased bark, freshly taken from the tree, into healthy trees as in budding. Later his inoculations were performed "with a sharp pointed knife or needle, dipped in the virus as it exuded from diseased trees, or which had been gathered and kept in a vial with distilled water."

J. C. Arthur (1885) cultivated the bacteria in sterilized infusion of corn meal for nearly four months and then inoculated the green fruit of Bartlett pear and produced the disease. The juices of blighted tissues, from which the bacteria had been removed by filtering through porcelain, failed to start the disease when introduced into green fruit. He concluded, therefore, that the bacteria are "the direct cause of the disease", thereby confirming the results of Dr. Burrill. Since that time, other contributions which deserve mention are the works of Waite (1898), Jones (1902), Whetzel (1906), Jones (1909), and Stewart (1913).

4. Hosts, Although the most important hosts of Bacillus amylovorus are the pear, apple, and quince, the disease also affects the plum (Jones, 1902), apricot (Paddock, 1903), prune (1915), crab apple, mountain ash, loquat species of Crataegus, Amelanchier canadensis, Pyrus americana, evergreen (Eriobotrya japonica), and red berried California holly (Heteromeles arbutifolia) (Waite, 1907).

5. Economic importance. Fire-blight is the most important disease affecting the pomaceous fruits. Because of the epidemic nature of the disease, it may be so virulent at times that entire orchards are

wiped out or pruned so severely that several years are required for recovery. These epidemics have been recorded as far back as 1844 when the disease was reported by Beecher (1844) to be widespread and destructive. Various epidemics have occurred since that time. One serious outbreak in Massachusetts about 1860 is recorded by Cooke (1867), and the most recent severe one appearing beyond the Rocky Mountains was reported by Pierce (1902) and Smith (1906) as occurring in the pear orchards of California where the pear industry was completely wiped out in certain sections. In the eastern states, pear blight has done serious damage. It is less severe in the northern states, but increases in severity toward the south, having caused great havoc in the orchards of New Jersey and Delaware. South of these states, Bartlett pear culture has been entirely abandoned except in a few localities in the mountains. The Oriental pears are somewhat more resistant and are grown in these southern states. The apple has suffered to a great extent in the eastern states, Mississippi Valley states, and more recently in the western states.

In New York, according to Whetzel (1906), injury in some sections of the state, notably the Upper Hudson River Valley, has been as high as 95 percent on trees between 8 to 15 years old, a very large percentage of the affected trees being dead or fast succumbing. Pickett (1914) reports that the disease in Illinois during certain years becomes a serious menace to the apple and pear industries of the state. In 1914, he places the damage in one county at \$500,000 and estimates the damage to the crop over the entire state at not less than \$1,500,000 for the season. Cranefield, Secretary of the Wisconsin State Horticultural Society, informed the writer that the

damage in Wisconsin runs up into the hundreds of thousands. A count estimate could not be given, but he stated that \$500,000 damage would be a low figure.

6. Causal organism, Bacillus amylovorus (Burrill) Trev. is typically rod shaped with rounded ends, although it sometimes appears oval in shape or almost spherical. The cells usually occur singly, but they are also often observed in pairs, fours, or even more, in chains, or in small clumps with their flagella matted together. The bacillus is 1 to 1.8 microns long and .6 to .8 microns broad. No endospores or capsules have been observed. They are usually actively motile by means of one to four flagella which are 6 to 12 microns long.

7. Life cycle. The bacteria remain alive during the winter in "hold-over" cankers, which will be fully discussed later in this article. These cankers become virulent with the ascent of sap and the warmth of spring. The bacteria multiply, spread into the adjoining healthy bark and, due to mass action of the invading organisms (Smith, 1911) combined with the osmotic pressure of the substance in which the bacteria are found, (Bachmann, 1913) exert a great pressure which ruptures the host cells and forces a bacterial ooze out of the lenticels or cracks of the infected regions. This gummy exudate which contains countless numbers of bacteria appears at blossoming time and serves as the primary source for blossom and twig infection. Waite (1906) lists two main points of entry of the organism: (1) the blossom clusters and (2) the tender tips of growing twigs. He also states that blight occasionally enters in a third way, that is, directly into the tender growing fleshy bark, although, as a general rule, this method of entry

is comparatively rare.

Insects such as honey bees, wild bees, wasps, flies, (Waite, 1906) boring beetle, or biting and sucking insects (Jones, 1909) are attracted to the sweet gummy exudate, become smeared with it and then in flying about infect other blossoms and twigs. The nectary of the blossom is not covered by cuticle, and is, therefore, the easiest place for the bacteria to enter. The gummy exudate pushes out of the infected blossoms into the nectar and this forms the chief source for secondary infection. Besides the insects listed above, the aphids (Jones, 1909) play an important part in spreading later infection to water sprouts and suckers, and cause all twig infection other than that due to blossom infection. Waite (1906) also believes that the fire blight organism is carried long distances on the feet of birds covered with this gummy material and by sap suckers puncturing hold-over blight, and transferring the disease to healthy trees. Other means of spreading the disease are by implements used in cultivation, ^{and instruments} such as the pruning knife, saw, chisel, shears or other workman's tools used in the orchard. These new infections occurring throughout the summer in a few cases form the hold-over cankers which carry the disease thru the winter.

III. APPEARANCE OF THE DISEASE

1. General appearance of the disease in the tree

Fire blight usually first attracts attention in the spring, a month or so after the blossoming period. At this time the flower clusters are shriveling and dying. The twigs are also affected at the same time, and in some cases, the new shoots are attacked at

the tip from which point the disease runs downward killing these twigs completely and spreading to other twigs and branches... During some seasons the diseased area extends only a few inches down the twig. On the other hand, if conditions are favorable for the blight organism or unfavorable for the tree, the disease may progress into the limbs and trunk of the tree, even into the roots.

On the apple, the disease occurs more often in the form of blossom and twig blight, while on the pear the blight is usually more severe in its affect upon the body of the tree. As a rule, only the young growth of the apple tree is destroyed by the disease and the tree survives the attack from year to year with a loss of vitality which in time results in unprofitable bearing. The disease, however, may cause death by spreading into the limbs, trunk, and roots of the tree as before stated. The bark of the pear is less resistant to the attacks of the organism than that of the apple and it is not unusual for a pear tree to be killed in one season.

2. Blossom blight. Blossom blight as a rule is responsible for the greater part of the infection in early spring. The first symptom of the trouble is a brown and later blackened appearance of the blossom clusters and the young leaf tufts. This form of blight prevents the development of the fruit and may spread down to the larger twigs and branches.

3. Twig blight. The disease in the twig is known as twig blight and occurs in blossom twigs, foliage twigs, water sprouts, and suckers. The characteristic appearance of this form of blight is a light or brown discoloration of the leaves and affected twigs.

The diseased leaves die, shrivel up, and remain attached to the affected twig often throughout the winter. This discoloration and death occur rather suddenly and may take place from blossoming time to September. The affected area of the twig later becomes dark, almost black, and shrunken. Very often the bark is blistered and from these blisters or pores the gummy fluid exudes. The bacteria may travel down the twig from $1/4$ of an inch to 2 inches in a day. Infection of the branches takes place from these infected twigs, often resulting in the girdling and death of the limb.

4. Body blight. Blight on the trunk or main limbs of the tree forms what is called "body blight". Almost all of these infections are due to infection from twigs, water sprouts, suckers, or wounds. The cankers or affected areas, vary in size from half an inch in diameter to as much as a foot or more in length and measure several inches across. On healthy vigorous trees they are small, more or less circular in outline, darker in color than the healthy bark, somewhat sunken, and the margin along the advancing front is usually slightly raised or blistered. On damp, cloudy days, drops of the milky, sticky exudate characteristic of the disease ooze out from the cankered tissues through the lenticels or pores in the bark. Older cankers are brown and distinctly sunken with a smooth surface. The margin of a diseased area is definite and is marked by a crack where, in drying, the diseased tissue has separated from the healthy bark. Humid atmosphere and cloudy days determine the progress of the disease. During the bright sunny days the progress of the canker is checked, but it starts to enlarge again with favorable weather. This condition

results in large cankers with concentrically arranged cracks within the cankered area. Superficial cankers are formed in cases where the organism never reaches the cambium but works entirely in the outer bark. Wounds of this character heal rapidly beneath the dead bark, which adheres to the tree as a scale. As a rule, the diseased bark is killed to the wood to which it clings the first season. It gradually decays, however, falls out, and leaves the wood bare.

According to Waite (1906) the exudate in the summer time from these cankers is milky white, later the gum oxidizes into an amber yellow, or slightly brownish, then finally into a dark brown, or almost black, gum. From the bark of large branches or bodies, it is often reddish brown from the start.

5. Collar blight or Root blight. According to Smith (1909) the most discouraging and baffling feature of the disease is where large numbers of trees in an orchard apparently free from blight are suddenly found to be affected in the butts and are thus beyond redemption by the usual methods of handling blight in the tops. Butt and later root infection takes place through green sprouts or suckers even when the latter amount to no more than a bud with only one or two leaves formed. He believes that infection is brought about by insects feeding on the green shoots. From the butt the disease progresses into the roots, and in time the death of the tree results. Waite (1906) noted that pears budded on French stock sprouted badly from the collar and that these sprouts became infected causing the loss of nearly every tree in an orchard. Pears alongside, under the same conditions, budded on Le Conte stocks had few sprouts from the collar and remained practically sound.

6. Fruit blight. The disease often attacks immature fruit, but is seldom found upon mature fruit. The bacteria find entrance thru the peduncle or skin punctures due to insect injury. According to Jones (1909) the diseased portion on green fruit is first light brown, but changes to a dark brown and finally to black. The flesh becomes soft and pulpy, and the skin wrinkled. The characteristic exudate often appears on the surface in the form of a slimy liquid. This slimy liquid is present within the apple tissue and is swarming with bacteria. In ripe infected fruit, the brown discolored areas are not slimy, soft, and pulpy, but contain many organisms. Infected fruit soon dries out, shrinks, becomes deeply wrinkled and turns black and hard. In this condition it remains on the tree over winter.

7. Microscopic examination of diseased tissues. Waite (1906) on microscopic examination found the diseased tissue to be completely filled with countless millions of the bacteria. These proceed in all directions from the point of infection, upward, downward, and laterally. The most rapid development is lengthwise, on account of the vessels and fibres in the bark. The bacteria invade the vessels of the bark, the intercellular spaces, and besides break down in their progress, the surrounding tissue which becomes filled with a mass of the gummy fluid. This frequently exudes wither in tiny drops or in the form of copious gummy exudate. Miss Bachmann (1913) found "the first evidence of infection to be a transparency around the point of inoculation followed later by a browning in the same region. She concluded that the transparency was due to the removal of air from the intercellular spaces, this being replaced by the liquid in which the bacteria live. The cells die because of a loss of

water". She states further that her experiments on the non-toxicity of the bacterial products on the host cells agree with the experiments of Arthur (1885) who found that the solution in which the organism grows, did not rot green fruits upon inoculation. The bacteria were found to be most abundant in the intercellular spaces, but, due to mass action of the bacteria and an osmotic pressure of the substance in which the bacteria are found, some cell walls are ruptured.

IV. LITERATURE REVIEW OF OVER-WINTERING

Various opinions have been set forth relative to the over-wintering of Bacillus amylovorus and considerable work has been done pertaining to this subject. A survey of published writings will be discussed in order to give a clearer understanding of the present situation.

According to T. J. Burrill of Illinois (1881) even the winter weather does not offer complete immunity to the trees. During two winters he reports having watched the slow progress of the disease in the branches of the same tree, and feels positive there was no mistake as to the nature of the phenomena.

Burrill (1882a) cites a case in which a nurseryman in Illinois experienced heavy loss due to blight in grafts. The work was done in mid-winter and old sawdust was used for packing material. The boxes were stored in a frost proof cellar. He relates further that "an examination clearly showed that the scions were at the time undergoing the destructive process we call blight". Pear limbs were inoculated with the bacteria laden fluid of the diseased scion, and the disease was produced. "The disease had plainly started from the cut ends of the scions and in most of these received had not yet involved the whole length".

Arthur in New York (1885) states that experiments have shown that the bacteria retain their vitality for a considerable time in limbs cut from the tree and thrown upon the ground. The most interesting results came from cultures started with the inner bark of a blighted limb that was cut from the tree in midsummer and thrown into a tank of water in the garden. In the fall the water was drained off, but the sediment remained moist till spring, the examination taking place the middle of April. After a period of nine months under these circumstances, the bacteria were found to be alive and in a growing condition.

"A solution was made by treating soil with boiling water, filtering, and sterilizing. This gave a nutrient solution in which the bacteria grew with moderate readiness, producing a pellicle and zoogloea. A culture of this kind started with bacteria from the quince, reproduced the disease in a Bartlett pear tree by inoculation.

He concludes by saying that, "the two facts that blight bacteria may retain their vigor in dead timber from one season to another, and that they can thrive in garden soil, make it evident that it is the part of cautious treatment to burn all blighted limbs when taken from the trees and to remove affected limbs before they drop the exudate that may convey the germs to the soil".

In a recent conversation with Dr. Arthur, he informed the writer that in his early work with Bacillus amylovorus he had confused the organism with ^aanother superficial saprophytic bacterium. In view of this statement, the above account may not be entirely correct in every detail. However, it is given to point out the general opinion advanced at that time.

Cassidy of Colorado (1888) states that in apple twig blight "the progress of the disease is much more rapid in warm weather, but it is known that parts infected enlarge during the season of rest.

Waite of Washington, D. C. (1898) is convinced that the pear blight organisms remain alive through the winter in those regions of the tree where the blight was active at the close of the growing season. "The bacteria remain alive along the advancing margin of the blighted area, and, although their development is very slow, it is continuous". He also adds that occasionally infections keep running down the twigs, get into the fleshy bark, and keep on working slowly till fall. After this season of the year, the trees are so moist that the organisms will not dry out, but will live over winter, resulting in what he calls hold-over blight. "In some instances in the South very active changes go on in these hold-over areas during mild weather, but in the northern states they do not have time to do much damage. In the spring, when the trees are full of sap, the bacteria that have outlived the winter start anew and extend their work".

Harrison of Ontario, Canada, (1904) states that "the organism is not killed by the winter frosts, but lives in the bark in a dormant condition until spring".

Waite (1906) observes that in Maryland he once saw a nursery block of 10,000 trees of Bartlett and other pears nearly completely destroyed by blight. This block, as was determined from the specimens, carried actual samples of hold-over blight in the stocks. When the stocks were cut off above the dormant buds in the spring, the pruning tools became infested and the disease was trans-

mitted to nearly every tree reached by the pruners.

Whetzel of New York (1906) states that "a large percentage of the cankers are active during but one season. There are always some, however, in which the disease is perennial, living thru the winter to become active again the following spring, spreading and enlarging the original limits of the cankered area".

Lawrence of Washington (1907) also states that "by the close of the growing season nearly all of the bacteria in the diseased tissues are dead. In a few areas, however, they continue to penetrate farther into the tissue. It is in this manner that some of them live during the winter, When the tree becomes active in spring the bacteria that have kept up a slow growth during the winter begin to grow at a much more rapid rate".

Jones, Ontario, Canada, (1909) says that the disease is much less active during the winter than during the summer, though the complete cessation of its activity during the cold period is questionable.

Sackett of Colorado (1909) holds that "by the middle of summer the active progress of the blight is checked by natural causes, but the disease is still present in what may be termed a latent form. In this stage it sometimes remains through the balance of the season and, protected by the bark of the twig or limb, it may live through the winter, giving rise to what is known as hold-over blight".

Smith, E. F., Brown, Nellie A., and Townsend, C. O. (1911) in bulletin on "Crown Gall of Plants" report that O'Gara in Oregon states that hold-over blight (B. amylovorus) is very apt to find lodgment in the galls when they occur above ground and that root rot

begins commonly in the galls when they are under ground. He also says that "anyone who has had experience on the Pacific Coast knows that a crown gall above the crown of a Spitzenberg means blight infection sooner or later and that during the past year he has seen hundreds of blight infections through these galls.

The best work pertaining directly to the hold-over blight in the pear has been done by Sackett in Colorado (1911). He began his investigation in the summer of 1909, and carried it on through the winters of 1909-1910 and 1910-1911. During the summer he tagged twigs varying in diameter from 1 mm. to 40 mm. ($3/8$ to $1\ 1/2$ inches) in which the blight was unquestionably present. Material from five different orchards was examined. These orchards were representative of practically 2000 square miles of fruit land on the Western Slope of Colorado.

Feb. 28, 1910, he collected hold-over material tagged during the summer of 1909. Out of twenty-one specimens examined, seven or 33.33 percent, contained Bacillus amylovorus. Three limbs between one and two and one-half inches in diameter contained no living blight organisms. "Ten of the samples varied in diameter from one-half to one inch and four more were water sprouts. The seven positive cases all occurred in the branches having a diameter of one-half to one inch, and four of these were small yearling twigs of 1909 growth".

The first collection for the 1910-1911 series was made on Jan. 26 and 27, 1911, from blighted material tagged during the preceding summer. The winter was regarded locally as open with considerable snow and rain. Twenty-eight samples were collected,

and from six, or 21.43 percent of these, virulent cultures of Bacillus amylovorus were obtained. These cultures produced the disease upon reinoculation.

April 12 and 14, 1911, the second series was gathered. At this time the pear buds had burst, but the blossoms showed no color. No exudate was observed from the blight cankers except in one orchard where abundant oozing was found on the larger blighted limbs. Thirty-four branches and twigs were examined from which eight or 23.53 percent, yielded virulent cultures of the blight organism. The cultures also produced the disease upon reinoculation.

Out of a total of 83 twigs examined, he found that twenty-one, or 25.3 percent, contained living Bacillus amylovorus. These twigs ranged from 7 to 30 millimeters in diameter. His cuttings were made from a zone just next to the living tissue. Sackett concludes that, "under Colorado conditions, at least 20 percent of hold-over cankers on the small limbs and twigs contain virulent blight organisms at the time of blossoming".

Smith of California (1911) notes that "the disease often runs down into the large limbs, where it remains alive over winter, producing the so-called hold-over blight, which is a source of infection during the following season. In the smaller twigs and branches the organism dries out and becomes entirely dead".

Swingle in Colorado (1911) states that "in the deep layers of the bark, in the line between the diseased and the sound tissues, the bacteria that cause the blight live over winter".

Hall in Washington (1913) notes that "upon the young fruit the blight causes a wilting of the tissues, then the fruit gradually dries up and if left upon the tree, forms an excellent hibernating place for the hold-over blight. In the warm, damp weather of the spring some of the old dried apples are found to be exuding a slimy substance that is full of the disease causing organisms. He states further that the blight is carried over from one year to another in the form of what is known as hold-over blight. This form may be found in any part of the tree that is affected with the disease, in cankers upon the trunk or large limbs, or even in the dead twigs that are left upon the trees.

Jackson in Oregon (1913) states that "cankers on the larger limbs and trunk may retain active bacteria over the dormant season and form the so-called hold-over cankers, which doubtless afford the only source of infection for the blossoms in the spring¹⁷.

Stewart in New York (1913) made isolations in March from diseased twigs (less than one centimeter in diameter) of Bartlett pears, taken from an orchard near Newark, New York and these gave about the same results as did those of Sackett (1911). An attempt was made to isolate the organism from five blighted twigs, and a pure culture of Bacillus amylovorus was obtained from two of these. The organism isolated produced the characteristic blight when used for inoculating the tender twigs of quince trees growing in the greenhouse.

In the same month, the fire blight organism was isolated also from pear seedlings that had blighted the previous season. The trunks of the diseased seedlings were about one-half inch in diameter.

Pickett in Illinois (1914) states that "in some cases the bacteria do not all die but continue to work very slowly into the surrounding tissues, prolonging the disease for several months or even over winter. These are the hold-over cankers, which carry the disease from one season to the next. The most serious of these hold-over cankers in apple trees are probably the larger ones which are found on the larger branches, in the forks of the branches, and in certain cases of "collar-rot" in large diseased areas on the trunks just above the surface of the ground".

V. CULTURAL CHARACTERISTICS OF THE ORGANISM

The cultural features have been fully worked out by Jones (1902), Jones (1909) and Stewart (1913), so that no work was done by the writer on this phase except to find out a suitable medium for work and to get acquainted with the cultural features on different media. Directions given by Smith (1905) were followed as closely as possible in the preparation of culture media. Fuller's scale was used in calculating the acidity and alkalinity.

1. Normal nutrient bouillon agar. On plates at room temperatures, 20°C., a transparent growth, one-fourth inch in diameter with entire margins, was obtained on the second day. A slight increase in growth took place up to the sixth day when it ceased. The colony was dry after the twenty-first day.

2. Nutrient bouillon agar -30. On plates at room temperature, 20°C., on the sixth day, a light growth about two inches in diameter spread over the agar. This growth was very thin, practically a film over the agar, and the colony was irregular with four branched arms. There was slight growth up to the sixth

day when the colony dried up.

3. Potato agar +13. Plain potato agar plates after the second day grew colonies with a raised, cloudy, growth one-eighth of an inch in diameter, with margins entire. On the fourth day there was only a slight increase in diameter of the colony, but a marked increase in thickness. Good growth, with raised edges, and with a diameter of three-fourths of an inch was obtained on the twelfth day. The eighteenth day, the colony was still healthy, with a grey center, and a white ring of growth on the edges, with entire margins. Growth was still apparent after the twenty-first day.

4. Potato agar +23. Agar acidified with lactic acid. After the second day there was only slight circular growth with entire margins. No growth was evident after the fourth day.

5. Potato agar +8 to +15. Good growth on agar slopes at room temperatures after the first day, slightly raised, glistening, semi opaque, with entire margins. The white streak did not branch on the surface nor penetrate the agar. During the next few days the growth became more raised, somewhat wider, more dense white in color, and spread out toward the bottom of the tube where moisture was present on the surface of the agar. By the seventh day the stroke began to dry out, becoming somewhat yellowish in color. No odor was noticed. The bacteria grow best under acid conditions between -10 and +15 Fuller's scale. Ordinary prepared potato agar with a normal acidity of +8 to +13 was used in all the culture work and proved to be entirely satisfactory. Cultures of organisms carried for five years were still virulent.

6. Normal nutrient bouillon. A uniform clouding was produced after twenty-four hours at room temperatures. The cloudiness was uniform, moderate, and no pellicle, ring, or odor was produced. After forty-eight hours the cloudiness increased and a sediment was produced.

VI. RESISTANCE TO DRYING

The resistance to drying of the blight organism is closely related to the question of over-wintering. Various opinions and experimental evidences have been given as regards the degree of desiccation the bacteria can withstand.

Probably the most extreme case is cited by T. J. Burrill of Illinois (1882 b) in which he states that a "drawing was made January 12, 1882, from material taken from a blighted tree October 1880, and kept dry on the point of a quill. When re-moistened the minute organisms seemed to be as lively as when first secured. A little iodine solution applied to them while under observation through the microscope at once stopped the movements, showing that the latter were truly vital activities". Burrill, however, did not perform any inoculation experiments to prove his point.

Waite of Washington, D. C. (1898) states that this organism is very delicate and forms no spores. It is readily killed by drying, and in the blighted twigs it dries out in one or two weeks when exposed to ordinary summer weather.

According to Jones of Ontario, Canada (1909) the resistance to drying was as follows: "Cover glass smears of broth cultures dried in the dark, at room temperatures, for five days, gave good growth in bouillon. Similar smears dried in bright

sunlight for thirty minutes gave growth in bouillon. Similar smears exposed to bright sunlight thirty minutes and kept dry in laboratory for six days gave no growth in bouillon".

An experiment to determine the persistence of Bacillus amylovorus in pruned apple twigs was carried on by Fulton of Pennsylvania (1911). He worked with blighted twigs under orchard conditions. "Four lots of blighted twigs of apples were cut and allowed to remain on the ground under different weather conditions from July to late September. Tests for viability were made by inoculating healthy twigs by bits of bark. Out of 35 twigs, 31 twigs, or about nine-tenths were inactive at the end of seven days, and the majority of these had become inactive in 3 to 5 days. There was indication that persistence was longer with low temperatures and moist conditions, than with high temperature and either dry or moist conditions. Bacteria in the dried exudate remained viable several days longer than bacteria within the tissues. The quick death of blight in cut twigs and the remote change of their being transferred to living trees, brings in question the necessity for rigid destruction of blighted twigs after removal".

Stewart of New York (1913) worked out the following results in his experiments on the resistance to drying. Tiny drops of a ten days-old peptonized beef bouillon culture were transferred to sterile cover-glasses in a covered sterile petri-dish and allowed to dry in the dark at a temperature of 20° to 22°C. The covers were then taken up by means of sterile forceps and dropped into tubes of sterile bouillon, one to each tube,

with the following results: number of days dried, 1, 2, 3, 4, 5, all cultures were alive and gave good growth in the bouillon tubes. The experiment was repeated, using a two-days-old peptone bouillon culture. The drops were about the size as in the previous tests. Covers were kept in the dark. The results were as follows. (These first tests were made after six days drying).

6 days	-	4 tubes	-	all good growth
7 "	-	2 "	-	" " "
9 "	-	2 "	-	no growth
12 "	-	2 "	-	" "
14 "	-	2 "	-	" "

In order to determine whether there was any difference in the resistance of the bacteria to drying under moist and dry conditions, and under cold and medium temperatures, various experiments were conducted. Test tubes were prepared with a piece of glass tubing about two and one-half inches long placed in the bottom of each. Half of the tubes were left dry and in the other half was put two to three centimeters of distilled water. They were then sterilized in an autoclave for thirty minutes at twenty pounds pressure. Cover slips cleaned in cleaning solution, then washed in water for a few hours, and rinsed in alcohol to be sure that no acids were left, were sterilized either by flaming and placing in sterile petri dishes in the transfer case, or sterilized in petri dishes in a hot oven. In one part of the experiment bouillon cultures of virulent bacteria were used for transfers. One platinum loop full of the culture was placed upon each cover slip, care being taken to so spread the bacteria that no lumps or groups of organism would collect upon drying. The en-

tire operation was done under sterile conditions in a transfer case. The drops of culture were then allowed to dry on the cover slips before transferring, with sterile forceps, to the sterile test tubes. Half of these test tubes, both damp and dry, were put in a graduate incubator at 1.2°C . and the other half at 16.4°C . Controls were made by putting a drop of the culture used, directly into a tube of sterile bouillon, and also by placing prepared cover slips upon drying into tubes of sterile bouillon. At intervals of twenty-four hours cover slips were removed with sterile forceps, under sterile conditions, and placed into tubes of sterile bouillon. Growth was then noted and recorded as shown in Tables 1 and 2.

Desiccation Experiment

1. Dry test tube. Bouillon culture.

Temperature	No. slips	No. days	Growth
1.2°C .	5	1	3
1.2°	4	2	3
1.2°	3	3	1
1.2°	4	4	4
1.2°	3	5	1
16.8°	5	1	1
16.8°	4	2	2
16.8°	3	3	2
16.8°	4	4	0
16.8°	3	5	1
Control	7	Put in bouillon after drying	6
Control	1 drop	Put in bouillon	Heavy growth

2. Damp chamber. Bouillon culture.

Temperature	No. slips	No. days	Growth
1.1°C.	5	1	1
1.1°	4	2	2
1.1°	3	3	0
1.1°	4	4	2
1.1°	3	5	1
1.1°	1	6	0
16°	5	1	4
16°	4	2	1
16°	3	3	0
16°	4	4	0
16°	2	5	0
16°	2	6	0
Control	7	Put in bouillon upon drying	6
Control	1 drop	Put in bouillon	Heavy growth

The results in table 1 show that growth was obtained in the dry test tubes after 5 days of desiccation, both at 1.2°C. and 16.8°C. A larger percentage of growth was obtained at the lower temperature, showing that the bacteria were more resistant to drying under cold conditions. Good growth was present in the controls.

Under damp conditions as shown in table 2, the organism remained alive at 1.1°C. until the fifth day, while at 16°C. growth took place only after two days of desiccation which also indicated that the bacteria were more resistant to drying under colder conditions.

A comparison of the two tables shows that the greater percentage of growth at both temperatures was obtained under the drier conditions. This is rather contrary to what would be expected, but enough moisture was present in dry test tubes to sustain life; while a saturated atmosphere was present in the damp test tube chamber which seems to be less favorable to the bacteria.

The above experiments were then repeated using sterile water cultures, made by transferring the organism from a potato agar culture. The bacteria were well distributed about the water culture by shaking and then allowed to stand for five minutes so that all the larger masses of bacteria would settle to the bottom. Transfers were then made to sterile cover slips as above described. As is shown by Tables 3 and 4 no growth was produced when the prepared cover slips were transferred upon drying to tubes of sterile bouillon.

Desiccation Experiment

3. Dry test tube. Distilled water culture

Temperature	No. slips	No. days	Growth
1.7°C.	3	1	0
1.7°	3	2	0
1.7°	2	3	0
15°C.	3	1	0
15°	3	2	0
15°	2	3	0
Control	11	Put in bouillon upon drying	0
Control	1 drop	Put in bouillon	Good growth

4. Damp chamber. Distilled water culture.

Temperature	No. slips	No. days	Growth
1.7°C.	8	1	No growth
1.7°	7	2	"
1.7°	4	3	"
1.7°	3	4	"
14.7°	8	1	"
14.7°	7	2	"
14.7°	6	3	"
14.7°	4	4	"
Control	11	Put in bouillon upon drying	"
Control	1 drop of culture	Put in bouillon	Good growth

No growth was obtained either in the dry or damp chamber or at the two temperatures indicated. The organisms were, therefore, killed a few minutes after drying when prepared from a water culture. In order to verify this difference of drying down from bouillon and water culture, cover slips were prepared in the same petri dishes, one-half from water cultures and the other half from bouillon cultures.. No growth was obtained from the slips prepared with water cultures, while growth was obtained from the slips prepared with bouillon cultures after five days of desiccation.

To be sure that the organism in water cultures was not killed by the presence of small amounts of ionic copper, or other metals from the distilling apparatus, rain water was collected in an earthen ware jar and sterile water cultures prepared from this. Table 5 shows that the bacteria, as before, were killed

upon drying.

Desiccation Experiment

5. Pure rain water culture.

No.	Date	No. min. dried	Results
1	5/14	45 min.	No growth
2	5/14	"	"
3	5/14	"	"
4	5/14	"	"
5	5/14	"	"
6	5/14	"	"
7	5/14	"	"
8	5/14	"	"
9	5/14	"	"
10	5/14	"	"
11	5/14	"	"
Control	5/14	1 drop put into bouillon.	Good growth.

The difference in drying from bouillon and water cultures can only be explained by assuming that a protective covering was formed over the bacteria after drying down in the drops of bouillon cultures.

These experiments show that the organism is extremely sensitive to drying. In practical conditions the method of infection must be one by which the bacteria are either put in contact with tender host tissue which is injured or by being carried to the nectaries where a suitable medium is found from where the bacteria can enter the host tissue directly. Exudate washed down the trees after a rain will not be a source of great infection for the bacteria will be killed immediately upon drying.

The resistance to drying in infected wood, which is entirely different than drying on glass slides, will be taken up in a future chapter.

VII. RESISTANCE TO FREEZING

The resistance to freezing also plays an important part in the over-wintering of Bacillus amylovorus. All the investigators agree that the bacteria can withstand almost any amount of freezing. Waite of Washington, D.C. (1906) states that the blight organisms "are wholly uninjured by any degree of cold likely to occur. Temperature of 40° below zero have no effect whatever. They may even be frozen in this temperature, but thaw out immediately when plunged into warm water, proceeding then with their activities uninjured. Cold regards their development, but prolongs their life".

According to Jones, Ontario, Canada (1909) the organism in freshly seeded agar plates placed in ice and salt freezing mixture at a temperature which varied from 0°C. to -10°C. grew well after being subjected to the above conditions for 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 20 hours. 50 percent were killed after the 20 hours of freezing.

Stewart of New York (1913) found that the organism was resistant to long freezing. "Freshly poured agar plates were made from a six-days-old peptonized beef bouillon culture. The plates were wrapped in paper and immediately placed in a tin can, which was imbedded in an ice-salt mixture in a granite pail. The temperature obtained by means of the mixture varied from -14° to -16°C. Two plates were removed at each of the following intervals: 3 hours, 4-1/2 hours, 7 hours, 23 hours, and in each case

the plates were immediately placed in the incubator at a temperature of 23°C . Colonies developed in abundance in all the plates. Plates subjected to the freezing temperatures for 23 hours, showed some retardation; however, there was practically no decrease in number of colonies that developed.

On Feb. 3, 1912, three bouillon tubes and 3 agar slant tubes with fresh transfers from an agar slant culture were placed in a quinine can containing a mixture of pulverized ice and salt. The can, being provided with holes in the bottom in order to allow the escape of water from the melting ice, was placed in a granite pail. The freezing apparatus was kept out of doors and the temperature of the mixture was recorded 3 times per day. The culture were re-iced twice each day, a new supply of salt (about 3 tablespoonfuls) also being added each time. The average temperature was about -14°C .; owing to extreme cold weather, however, it was as low as -28°C . on one or two occasions. After 5 days of constant freezing, one agar and one bouillon tube were removed. A very slight growth was apparent on the agar slant, and on melting the bouillon it showed a faint cloudiness. The tubes were placed in the incubator at a temperature of 23°C . and after 18 hours agar plates were poured, in which developed the characteristic fine blight colonies. On the eleventh day of freezing 2 more tubes were removed and placed in the incubator. After 36 hours, growth was apparent in both the agar and the bouillon tubes. The last two tubes were removed from the freezing mixture on the 14th day and they gave good growth after 36 hours at 23°C .

During the winter of 1914 and 1915 in Wisconsin, the organism withstood the most severe weather while in culture. Potato agar slopes, potato agar plates, and bouillon cultures were subjected to the outside cold. These cultures were placed within a loosely closed paper box, to protect from snow, and exposed to the cold outside of an east window of the laboratory. Young and old cultures of bacteria were used in order to get the difference, if any, of resistance to freezing due to age of the colony. Potato agar slopes were prepared by placing thereon a loop-full of bacteria. Plates were poured by the dilution method and the bouillon tubes were prepared by transferring one loop full of a bouillon culture of bacteria.

Table 6. Resistance to Freezing.

Cult.	No. subjected	Days frozen	Temperature		Growth at room temperature
			Max.	Min.	
Slope	1	2	13°	-2° F.	V. good.
2	1	4	16°	-23° F.	"
day	1	3	16°	-19° F.	"

Slope	1	11	36°	-8° F.	Good
4	2	12	37°	-23° F.	Good in one,
day	1	13	42°	-8° F.	other dry
	2	14	45°	-23° F.	Good
	1	15	45°	-8° F.	"
	2	83		-8° F.	Dry, no growth

Slope	2	2	13°	-2° F.	Good
28	1	6	16°	-23° F.	"
day	2	3	16°	-19° F.	"
	2	4	16°	-23° F.	"

Plate	1	2	16°	-2° F.	30 colonies
2	1	6	16°	-23° F.	15 "
day	1	3	16°	-19° F.	7 "

Plate	1	2	16°	-2° F.	25 "
28 day	1	3	16°	-19° F.	2 "

Bouillon	1	2	16°	-2° F.	None
2	1	3	16°	-19° F.	"
day	1	4	16°	-23° F.	"
	1	3	28°	16° F.	"

Cult.	No. subjected	Days frozen	Temperature		Growth at room temperature
			Max.	Min.	
Bouillon	1	2	16° to	-2° F.	None
28	1	3	16° to	-19° F.	"
day	1	4	16° to	-23° F.	"

Table 6 shows that the agar slopes with two day old cultures withstood cold for at least four days during which time the temperature stood between 10° and -23° F. Agar slopes with four day old cutting withstood cold for at least 15 days during which time the temperature range was between 45° and -23° F. Agar slopes from twenty-eight day old cultures withstood this same cold for at least 6 days while the temperature stood between 16° and -23° F. Agar plates prepared with the two day and twenty-eight day old cultures produced twenty-five to thirty colonies after being subjected to temperatures between 16° and -23° F., and then brought into room temperatures. No growth was obtained after freezing the bouillon cultures except in one case which was subjected to a temperature between 28° and 16° F. The two agar cultures kept out 82 days at a temperature ranging between -8° and 45° F. failed to grow after being brought into room temperatures. This failure of growth was due to drying out rather than effects of prolonged freezing.

These results show that the blight organism is uninjured by any degree of cold which is likely to occur. No difference was found in the susceptibility to cold between old and young colonies.

VIII. TEMPERATURE RELATIONS

1. Growth in graduate incubator and at various other temperatures from 1°C. to 35°C. Temperature relations were determined by subjecting the cultures of bacteria, both on agar and in bouillon, to a range of temperature between 1°C. and 16°C. in a graduate incubator, as well as at room temperatures 20° to 23°C., in incubator at 24°, 31°, and 36°C., in the ice box at 9°C., and in the cellar at 8°C. In these experiments transfers from two, twenty, twenty-four, and twenty-eight day old cultures were used to find if there was any difference in growth at various temperatures due to age of the bacteria.

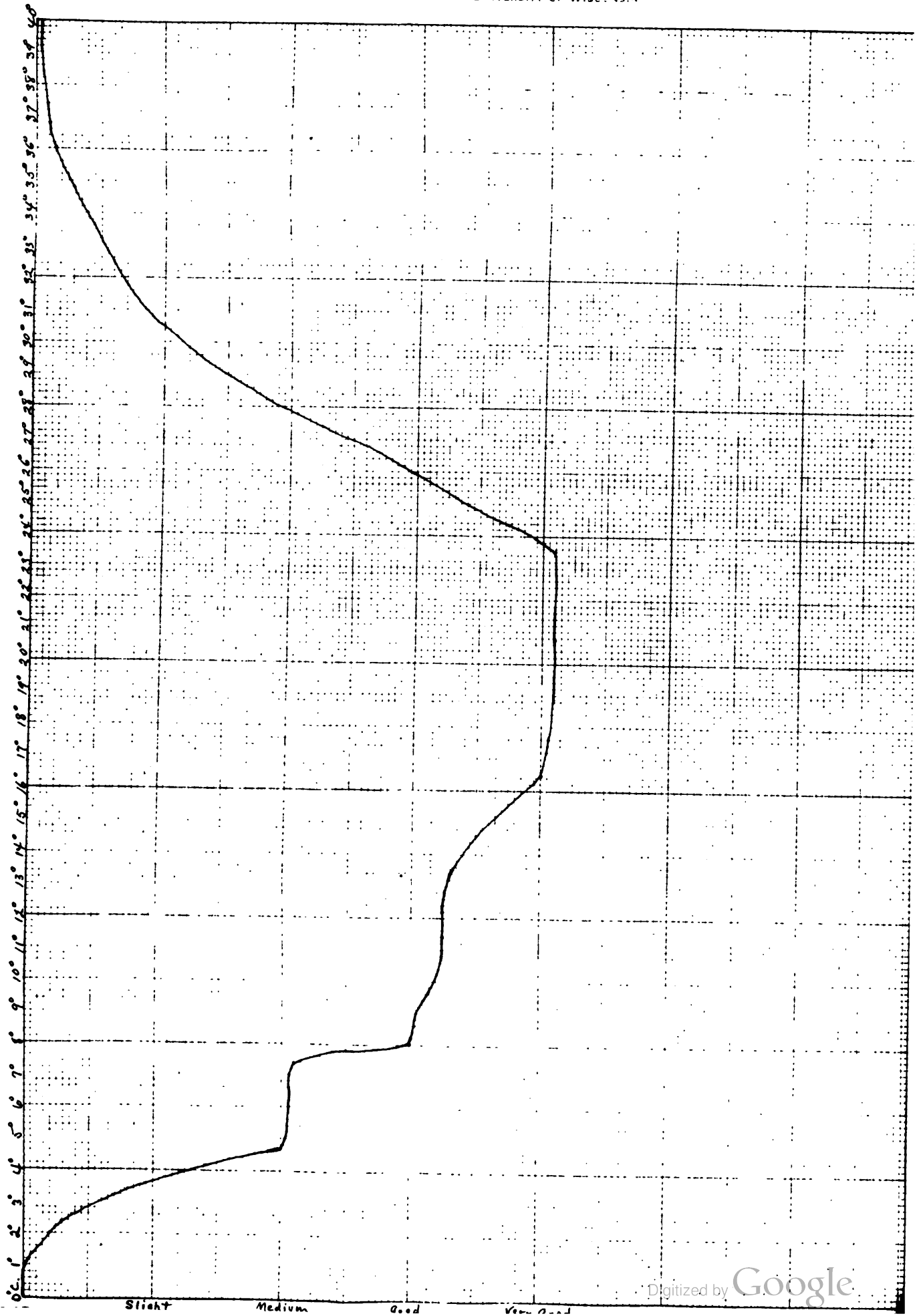
Table 7. Temperature Relations

Temperature	2 day old	20 day old	28 day old	Bouillon 24 day old
1.°C. Inc.	None	None	None	None
2.33 "	Very slight	"	Very slight	"
4.70 "	Good	Medium	Good	Medium
7.68 cellar		"		"
7.78 inc.	Good	Good	Good	Good
8.06 cellar	"		"	Medium
9.06 ice box	"	None	"	Slight
9.78 inc.	"	Good	"	Good
11.48 "	"	"	"	"
12.69 "	"	"	"	Medium
13.7 "	"	"	"	"
14.64 "	Medium	"	Medium	"
16.48 "	V. Good	V. Good	V. good	V. good
20.77 room	"	"	"	"
23.67 inc.	"		"	Medium
30.57 "	Slight	Slight	Medium	"
36.01 "		V. slight		None

Temperature Relations

FORM C2

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2. Growth on potato agar slope. Table 7 and the accompanying curve give the comparative growth at different temperatures. No growth was obtained down near the freezing point, but in one instance very slight growth was obtained at 2.33°C . From that temperature on upward growth kept on increasing until it reached the optimum between 16.5° and 23.7°C . As the temperature rose above 23°C . growth was not so good and at 30.5°C . it practically stopped. Slight growth was obtained at 36°C ., but the bacteria dried up in the course of a day. Observations were made at these various temperatures for a period covering twenty to thirty days. At the lower temperatures between 2.3° and 9.8°C . growth started between the second and fifth day, it being slight at first and reaching its point of best growth between the fifteenth and twentieth day. Between 9.8° and 14.6°C . growth started out earlier. It attained its height between the seventh and fifteenth day and then fell off and dried up. Growth at 14.6° and 20.8°C . was at its maximum the first few days. Then it gradually dried up. At the higher temperatures, 30° to 36°C . only slight growth was obtained and the culture of bacteria dried out in the course of a day. Stewart (1913) determined the death point to be about 47°C . when exposed for ten minutes in +15 peptonized beef bouillon.

3. Growth in bouillon. The table further shows that growths in bouillon and upon the potato agar slopes behaved about the same. In the bouillon, growth held on longer at the higher temperature, due to the fact that the organism was not subjected to desiccation combined with high temperature.

4. Comparison of growth of old and young cultures at various temperatures. No difference could be found between growth of young and old cultures. Cultures failing to grow at the lower temperatures grew when placed at room temperatures except when destroyed by long periods of drying. The bacteria in culture, therefore, grew very little or not at all at the low temperatures, but were not destroyed at these temperatures. When the temperature reached 2° to 4°C. above freezing growth started to take place rather slowly but increased with an increase in temperature until a higher temperature above 30°C. was reached. In nature the organism seems to behave in very much the same way, growing very little or not at all during the cold parts of winter, increasing in growth as spring advances, and at temperatures between 16° and 23° growing at its best. When hot weather sets in the advance of the disease is stopped. Later in the season, if the bacteria have survived the hot drying weather, growth and advance of the disease may start up again. Bacteria which have thus passed the hot summers, during fall, in a few cases, find suitable places for growth until winter sets in. As cold weather approaches growth is retarded, the bacteria remain dormant, and if the drying is not too severe they can survive the winter in the dormant condition and resume growth when favorable weather returns in the spring. In Wisconsin, as will later be explained, this method of over-wintering is rather rare, but a few cases of hold-over blight must be present during the normal winters.

IV. GREENHOUSE INOCULATION EXPERIMENTS

1. Introduction. Greenhouse inoculation experiments were carried on in order to determine the behavior of Bacillus amylovorus in apple seedlings; to observe the progress of the disease within the apple wood; to determine the length of time for the appearance of the disease after inoculation; the rapidity of spread; and the appearance of exudate. After the disease had run its course the infected seedlings were used for experiments to determine the persistence in cut and dried wood, and in seedlings matured by drying in pots to represent conditions taking place in nature. Seedling trees one year old were used for this work.

2. Inoculation of seedlings. Inoculations of seedlings were made by placing, with a sterile glass rod, one drop of a bouillon culture of bacteria in the axil of a leaflet in the young growing tip of a plant, and then pricking the seedling through the drop of culture with a sterile needle. A bell jar was placed over the entire plants and left for a few days until infection took place. During sunny, hot days, the bell jars were covered with cloth to protect the plants from the sun. In a few inoculations during cloudy days the bell jars were dispensed with. Controls were made in the same manner except that sterile water blanks were used in place of the bouillon culture of bacteria.

3. Observation of disease appearance. Observations were made daily. The first indication of infection was observed the second day after inoculation when a slight browning of the green shoot was noticed. On the third day the disease progressed

still further and by the fourth day, in very succulent growth, one inch of the seedling tip was infected, being slightly browned and drooping. A few days later indications of the disease were present upon the petioles even running up into the mid rib and larger veins of the lesser. The leaves then drooped and turned brown. By the seventh day after inoculation the disease in severe cases often ran down the tender shoots as far as four inches. If the tender shoots were only a few inches long they were entirely killed in the course of seven to fifteen days and the disease sometimes started into the older wood. In the majority of cases the older resistant wood hindered the progress of the organism. At the end of twenty-one days, however, in a few cases the infection extended from three to four inches into the old wood.

A bacterial exudate appeared on some of the infected twigs, the eighth day after inoculation. In the older wood a cracking was first noticed at the point of infection and out of these cracks the sticky, milky drops of exudate appeared. An exudate was also evident along the infected petioles and tender shoots. This exudate at first appeared clear, but in a few days turned brownish due to oxidation.

In all of these infection experiments 98 percent of the inoculations were effective. No infections were obtained by spraying a suspension of the bacteria upon plants and then covering with a bell jar. Inoculations made on a dark day without the use of bell jars produced 100 percent infection. All controls remained healthy. Plate I shows inoculated and control seedlings.

4. Exposing diseased seedlings to various temperatures and drying conditions. These inoculated seedlings were then sub-

jected to various different conditions before reisolation in order to determine the persistence of the organism in matured apple twigs and in pruned apple twigs. Diseased seedlings were left standing in the greenhouse and some were cut off and placed in a box outside under various weather conditions. Others were cut off and placed in the cellar, in the ice box, and in a desiccator. Potted diseased seedlings were allowed to dry out in the greenhouse and down cellar. At various intervals isolation of the organism was attempted from these infected twigs.

Table 8. Diseased seedlings exposed to various temperatures and drying conditions

Date inoo.	Extent of disease	Condition subjected	Temp.		Plated	Days subjected	Proof of
			Min.	Max.			
1/12/'15	Start in old wood.....	Left standing in greenhouse.....	18°	-22°C.	2/11/'15	30	+
3/ 4/'15	2 start new. shoot.....	Potted seedlings dried in green-house 4/26/15...	10°	-30°C.	6/ 3/'15	38	+
3/26/'15	Start in old wood.....	Ditto.....	10°	-30°C.	5/26/'15	30	+
2/13/'15	Ditto.....	Cut off, placed outside 3/15/15	16°	-51°F.	4/30/'15	46	+
2/18/'15	Ditto.....	Ditto.....	16°	-51°F.	4/30/'15	46	+
2/22/'15	Ditto.....	Ditto.....	16°	-51°F.	3/29/'15	12	+
3/18/'15	1-1/2" new shoot.....	Cut off, placed outside 4/26/15	36°	-80°F.	5/27/'15	31	+
4/16/'15	4" new shoot	Potted seedlings dried in cellar 5/3/15,.....	12°	-16°C.	6/ 3/'15	31	+
4/19/'15	1" new shoot	Cut off, placed in cellar 5/13/15....	12°	-16°C.	5/13/15	0	+

Table 8 (continued)

Date inoc.	Extent of	Condition subjected	Temp.		Plated	Days	Proof of
			Min.	Max.			
4/23/'15	2" new shoot	Cut off placed in ice	10°	-10°C.	5/26/15	16	+
		box 5/10/15.....					
4/24/'15	2-1/2" new.. shoot.....	Ditto.....	10°	-10°C.	6/ 3/15	24	+
4/26/'15	2" new shoot	Ditto.....	10°	-10°C.	6/ 3/15	24	+
4/28/'15	1-1/2" new.. shoot.....	Ditto.....	10°	-10°C.	5/20/15	10	+
4/26/'15	2" new shoot	Cut off, placed in... desiccator 5/20/15...	20°	-24°C.	5/26/15	?	+

(a). Diseased seedlings left standing in the greenhouse. As shown in table 8, seedlings inoculated January 12, and left standing in the greenhouse for thirty days, until February 11, still contained the organism. The disease in the wood by this time had entirely ceased to advance and in five twigs it was present one-fourth of an inch in the old wood. In five twigs the disease had advanced one-fourth of an inch in the old wood, in three twigs it had just started in the old wood, and in one twig the new shoot was slightly infected. Of these nine twigs, six were found to contain the organism. This preliminary test showed that the bacteria were not destroyed in the twigs thirty days after inoculation, the greatest part of which time they were in a semi-dormant state.

(b) Diseased twigs cut off and placed outside. Twigs inoculated February 13, 18, and 22, were cut off and placed outside March 15. Table 8 shows that the blight organism was isolated from some of these twigs 46 days after such treatment. The twigs were placed in a box so as to be protected from rain and sun on a north side of a building. During this period the temperature ranged between 16° and 51°F. Severe freezing at night took place, but during the greater part of the experiment the mid-days warmed up to a considerable extent. The disease had progressed into the old wood in most of these twigs. Seedlings in which the disease had advanced one and one-half inches and which were cut off and placed outside April 26, contained the organism after 31 days of drying. During this period the days were warmer having a temperature between 35° and 80°F. Hot, dry weather, therefore, has a tendency to lessen the life of the

organism in pruned diseased twigs. Diseased twigs left out in the orchard during the hot summer will probably not contain the organism for such a long period of time.

(c). Diseased twigs cut off and placed in cellar.

Table 8 shows that seedlings in which the disease had advanced one inch in the new shoot contained the organism when placed in the cellar. Isolation made seven, thirteen, and twenty-one days after subjecting to cellar conditions failed to show the presence of the blight bacteria. This was probably due to the fact that saprophytes got into the twigs and either destroyed or outgrew the blight organism.

(d). Diseased seedlings cut off and placed in ice box.

Diseased seedlings cut off and placed in the ice box, at a temperature of 10°C. still contained the organism after 24 days, as shown in Table 8. The disease had advanced one to two inches into the new shoot and seemed to stop at that point. A longer period in the ice box could not be obtained due to running out of infected twigs. However, from the work done it is rather to be expected that the organism may remain alive much longer under these conditions than the table indicates.

(e). Diseased seedlings cut off and placed in desiccator. Infected twigs placed in the desiccator at room temperatures, 20-28°C. still contained the blight organism after seven days of such treatment. An ordinary calcium chloride desiccator was used for this experiment and at the end of a few days the twigs were so dry that they became brittle, but still contained the bacteria. This result would be expected, for the organism

will remain alive for at least five days after drying down from a bouillon culture. Drying down in the sappy wood is somewhat analagous to drying down in a bouillon culture, hosever, with an added protection of the wood. A longer period of drying would undoubtedly destroy the organism.

(f).Potted seedlings left to dry in greenhouse. Instead of cutting off infected twigs, seedlings were allowed to mature in as natural condition as possible by drying down in pots in the greenhouse. Table 8 shows that this was done at a temperature between 10° and 30°C . The potted seedlings were taken out of the direct rays of the sun by placing under the benches. Such seedlings still contained the organism after 38 days of drying. No advance of the bacteria was made in the twigs except during the first two weeks. This experiment indicates that the organisms in the young twigs in the orchard only advances so far and then ceases to develop, lie in a dormant state for a period and are then destroyed by the hot drying condition found during summer.

(g).Potted diseased seedlings left to dry down cellar. Table 8 shows that the seedlings infected four inches in the new shoot and which were placed down cellar at a temperature between 12° and 16°C . still contained the organism after 31 days of treatment. The results obtained down cellar compare rather favorably with those of the potted seedlings dried in the greenhouse. It is to be expected, however, that the bacteria may remain alive longer under a cool and moist condition, if no saprophytic organism gains entrance.

5. Isolation of organism from the infected twigs after above treatment. The apparatus used for isolations consisted of three clear, sharp scalpels, one pair of forceps, and a small flask of alcohol. The scalpels and forceps were carefully flamed by dipping into alcohol and igniting. Infection twigs were first carefully washed and then immersed in mercuric bichloride for five minutes. They were then washed with distilled water and placed in the transfer case ready for isolation. The cuttings were made so as to include both infected and apparently healthy wood at the line of demarcation of the disease. The outer bark of a twig was removed with a scalpel which was flamed after each cut. A second flamed scalpel was then used to make cuttings. A ring was cut around the base of a prepared section of the twig and slices of bark cut down almost to the ring. With a flamed pair of forceps these bits of bark were then removed, under aseptic conditions, either on to plates of poured potato agar or into sterile bouillon cultures. If bouillon cultures were employed, poured plates of potato agar were made from these as soon as the organism develops, and transfers made from the predominating colonies produced in the plate. After many trials it was found that the cultures obtained by placing the bits of bark directly upon agar plates and transferring the bacteria to potato agar slopes, were in practically all cases single cultures of bacteria, and consequently this method was used in the majority of isolations.

X. ISOLATION AND OBSERVATION OF ORGANISM
IN DISEASED TREES IN THE ORCHARD

1. Isolation from infected twigs, water suckers, and body cankers. In order to determine whether any bacteria were living in the infected portions of the tree, isolations were attempted from twig, water sucker, and body blight. These isolations were started the middle of October, 1914, and carried on through the winter and into the spring of 1915. As can be seen from Table 9, infected twigs were collected from three localities. Isolations were made as described in a former chapter. In nearly every case a fungus or saprophytic bacterium was obtained in these isolations, but in no case could the blight bacteria be obtained. Out of the 154 isolations, 54 were cases of body blight and the remainder twig and water sucker infections.

Table 9. Isolation from diseased twigs in orchard

Date collected	Date plating	No. culture	Organism	Proof
Oct. 17, 1914	Oct. 19, 1914	1	Yellow	-
		2	White	-
		3	Yellow	-
		4	Yellow	-
		5	Fungus	-
Oct. 25, 1914	Oct. 27, 1914	6	"	-
		7	"	-
		8	"	-
		9	"	-
		10	"	-

Table 9 (continued)

Date collected	Date plating	No. culture	Organism	Proof
Oct. 25, 1914	Oct. 27, 1914	11	Fungus	-
		12	"	-
		13	"	-
		14	"	-
		15	"	-
Nov. 10, 1914	Nov. 10, 1914	16	"	-
		17	Yellow	-
		18	"	-
		19	"	-
		20	"	-
		21	"	-
		22	"	-
Nov. 12, 1914	Nov. 14, 1914	23	None	-
		24	"	-
		25	"	-
		26	Yellow	-
		27	Fungus	-
		28	"	-
		29	Yellow	-
		30	"	-
		31	"	-
Nov. 12, 1914	Nov. 18, 1914	32	White	-
		33	"	-
		34	"	-
		35	"	-

Table 9 (continued)

Date collected	Date plating	No. culture	Organism	Proof
Nov. 20, 1914	Nov. 20, 1914	36	White	-
		37	"	-
		38	"	-
		39	"	-
		40	"	-
		41	Sterile	-
		42	White	-
		43	"	-
		44	"	-
		45	"	-
		46	"	-
		47	Yellow	-
Nov. 25, 1914	Dec. 2, 1914	48	White	-
		49	"	-
		50	"	-
		51	Sterile	-
		52	White	-
		53	Yellowish white	-
		54	White	-
		55	White yellow	-
		56	White	-
		57	White yellow	-
		58	" "	-
		59	White	-
		60	"	-

Table 9 (continued)

50

Date collected	Date plated	No. culture	Organism	Proof
Nov. 25, 1914	Dec. 2, 1914	61	White	-
		62	"	-
		63	White & yellow	-
		64	White	-
Dec. 3, 1914	Dec. 7, 1914	65	Yellow	-
		66	Sterile	-
		67	Yellow	-
		68	"	-
		69	"	-
		70	"	-
		71	"	-
		72	"	-
		73	Yellow white	-
Dec. 17, 1914	Dec. 17, 1914	74	Fungus	-
		75	"	-
		76	"	-
		77	"	-
		78	"	-
		79	"	-
Jan. 2, 1915	Jan. 2, 1915	80	Yellow	-
		81	White	-
		82	"	-
		83	Yellow	-
		84	Pink and white	-
		85	Yellow	-
		86	"	-

Table 9 (continued)

Date collected	Date plated	No. culture	Organism	Proof
Jan. 2, 1915	Jan. 2, 1915	87	White and yellow	-
		88	Yellow	-
		89	White	-
		90	Yellow	-
		91	Yellow and white	-
		92	Sterile	-
		93	Yellow	-
		94	Fungus	-
		95	"	-
		96	"	-
		97	"	-
		98	"	-
		99	Yellow	-
		100	Fungus	-
		101	"	-
		102	Yellow	-
		103	"	-
		104	Fungus	-
Feb. 18, 1915	Feb. 18, 1915	105	Yellow	-
		106	"	-
		107	White	-
		108	"	-
		109	White and yellow	-
		110	White	-
		111	White & purple	-
		112	" " yellow	-

Table 9 (continued)

Date collected	Date plated	No. culture	Organism	Proof
Feb. 18, 1915	Feb. 19, 1915	113	Salmond	-
		114	White	-
		115	Yellow	-
		116	White, agar purple	-
		117	Fungus	-
		118	Yellow	-
		119	Sterile	-
		120	White & yellow	-
		121	Yellow	-
		122	White & yellow	-
		123	Yellow	-
		124	White	-
		125	White, agar purple	-
Feb. 18, 1915	Feb. 19, 1915	126	Sterile	-
		127	Yellow	-
		128	White & yellow	-
		129	Fungus	-
Apr. 15, 1915	Apr. 19, 1915	130	Yellow	-
		131	"	-
		132	"	-
		133	White & yellow	-
		134	Yellow	-
		135	"	-
		136	"	-
		137	"	-
		138	"	-

Table 9 (continued)

Date collected	Date plated	No. culture	Organism	Proof
Apr. 15, 1915	Apr. 19, 1915	139	White	-
		140	Yellow	-
		141	"	-
		142	"	-
		143	"	-
		144	White	-
		145	Yellow	-
		146	"	-
		147	Fungus	-
		148	"	-
		149	White	-
May 12, 1915	May 13, 1915	150	"	-
		151	Yellow	-
		152	White	-
		153	Yellow	-
		154	Sterile	-

Nos. 1-79 collected in U. W. orchard.

Nos. 80-104 collected at end of Mills Street.

Nos. 105-154 collected in Vilas Park.

Nos. 61-64, 100-104, 109-154 were body blight on branches from
1 to 6 cm. in diameter.

The remainder were twig infections on twigs from 3 mm. to 1 cm.
in diameter.

2. Marking of twig infections and body cankers. During the fall of 1914, blighted trees in the University orchard were carefully studied. In one portion of the orchard there was a seat of infection which covered twelve rows running each way. These trees were eight years old kept under clean culture on a north slope facing Lake Mendota. Various varieties were present in this lot, but the blight seemed to be most severe on Tallman's Sweet. From outward appearances one Tallman's Sweet tree, No. 3-9, which was badly blighted, seemed to be the source of infection for the neighboring trees. In order to watch these cases of blight in the most scientific way, cases of twig and body blight were marked in these infected trees. The line of demarcation between healthy and diseased wood was marked with India ink on 20 cases of body blight and 70 cases of twig blight as shown in Table 10.

Table 10. Infected twigs and body blight marked in orchard

Date marked	No.	Variety of tree	Body blight	Twig blight	Cases of hold-over blight
Nov. 25, '14	4-12	Tallman's Sweet	4	19	None
Nov. 25, '14	4-11		8	25	"
Dec. 3, '14	3-1	Newells Winter	1	5	"
Dec. 3, '14	6-8	McMahon	4	6	"
Dec. 3, '14	6-10	2-yr.-old		1	"
Dec. 3, '14	3-9	Tallman's Sweet	3	14	"

3. Observations of these marked lesions. During the winter observations were made to see whether or not the disease had advanced beyond the ink marks, but no advance was noticed. In the spring of 1915 no indications were present to show that the bacteria were still alive, as no advance was made in the extent of the canker and no bacterial exudate was present.

4. Close observation of diseased trees for appearance of the organism in the form of an exudate during spring. Observations were not only made of these marked lesions but hundreds of other infections were examined daily in different orchards. In no case could any evidence be found that bacteria were still alive. No exudate could be observed from the largest cankers or small twig lesions. One month after blossoming no new infections of blight could be detected in any of the trees observed, which were badly blighted in the spring of 1914. Still further evidence against the theory of living organisms in the young twig infections of last year is that new healthy shoots have started out at the margin of the diseased area as shown in Plate 2. Plates 3 and 4 represent types of body cankers observed and from which isolations were attempted. Plate 2 represents a blighted twig, collected the fall of 1914, with a healthy shoot extending from the base of the infected portion thus indicating that the blight organism in this case, as well as hundreds of other cases of twig blight, had been destroyed during the hot summer.

Although in other states investigators have found that Bacillus amylovorus remain alive over winter in the larger body cankers of the apple tree, the method of over-wintering under Wisconsin conditions as yet remains unsolved.

Bacillus amylovorus forms no spores, is very sensitive to drying, and therefore dies rapidly in the blighted tissues as soon as they become fully dried. Organisms from water cultures are destroyed immediately upon drying on a glass slide, while in drying down from bouillon cultures a protective film forms over the bacteria which keeps them alive up to five days. Desiccation within the diseased tissue is entirely different from that upon glass slides. Experimentation with blighted twigs shows that the plant sap protects the bacteria from rapid drying, that the weather conditions play an important part in desiccation, and that the organism is relatively short lived in the twigs after they are cut off from the seedlings or are left in the pots and allowed to mature by slowly drying. If the drying out of plant sap is not too severe in the diseased twigs, the bacteria will remain alive near the demarcation of the canker. It was found that during cold weather when the thermometer ranged between 51° and 16° F., that is when it was freezing at night and warming up slightly during the day, drying went on rather slowly and Bacillus amylovorus was obtained from cut twigs, placed in a box to protect from rain, 46 days after being put out to dry. Fulton (1911) obtained results on the persistence of Bacillus amylovorus in pruned apple twigs during the summer which showed that the bacteria became inactive in 3 to

5 days. No difference in the persistence of the bacteria could be noted when dried in the cellar and ice box compared with those dried outside or in the greenhouse. When exposed outside during cold weather, the bacteria lived longer than when exposed during hot weather. Blighted twigs dried down in a desiccator, contained living bacteria up through the seventh day of desiccation, thus corroborating the work done on drying bacteria down from bouillon cultures.

During the winter of 1914-1915 in Wisconsin, the most severe weather had no effect upon the life of the organism in culture if there was plenty of moisture present to keep the bacteria from drying out.

Experiments on the temperature relations seem to indicate that there must be some blighted area which contains the required amount of moisture to keep the bacteria from drying out and thereby tiding over the winter. Investigations in the field showed that during the winter of 1914-1915 no organisms remained alive in the twigs blighted the last season. Fifty twig isolations made in October and November without obtaining the organism, indicated that the bacteria were destroyed during the summer. This conclusion seems to be corroborated by the experiments carried on in relation to the persistence of blight in infected twigs dried under various conditions. Seventy twigs marked in the fall of 1914, as well as hundreds of others observed, showed no advance of the disease during the winter and no bacterial exudate in the spring. Two other possible places of over-wintering in the tree are in water suckers and in the trunk and larger

branches. Isolations failed to show the presence of Bacillus amylovorus in either case. Twenty well developed cases of body blight of last season were marked in the fall of 1914, on trees in the University orchard. Observations of these and hundreds of others, during the winter and spring showed that in no case had growth started in the cankers, or that a bacterial exudate was present. These results seem to indicate that blight this year in the orchards inspected will be of relatively slight importance.

There is a possibility that the organism will develop late in spring, although, according to other investigators, the bacteria should be present during flowering time. From the work already done, it would seem that an investigation covering the entire year and extending over a larger area is necessary in order to come to any definite conclusion concerning the overwintering of Bacillus amylovorus under Wisconsin conditions.

1794. Denning, William. On the decay of apple trees. Trans. New York Soc. Prom. Agr. Arts and Manfr. 1: 219-222.
1844. Beecher, H. W. The blight in the pear tree: its cause and a remedy for it. Mag. Hort. 10: 441-456.
1867. Cooke, S. S. Pear blight. Gardeners Monthly 9: 73-78.
1879. Burrill, T. J. Fire blight. Trans. Ill. State Hort. Soc. 12: 77-81.
1881. Burrill, T. J. Pear and apple tree blight. Trans. Ill. Hort. Soc. 14: 157-167.
1882. Peffer, Geo. Apple tree blossoms blight. Trans. Wis. Hort. Soc. 12: 191-202.
- 1882a. Burrill, T. J. Have we any new light on pear blight or yellows? Rept. Mich. State Hort. Soc. 1881: 133-139.
- 1882b. Burrill, T. J. Bacteria and their effects. Pear blight. Trans. Ill. State Hort. Soc. 15: 179.
1885. Arthur, J. C. Proof that bacteria are the direct cause of the disease in trees known as pear blight. Proc. Amer. Assoc. Adv. Sci. 34: 295-298.
1887. Arthur, J. C. Pear blight. Rept. N. Y. (Geneva) Agr. Exp. Sta. 5: 275-289.
1888. Cassidy, James. Apple twig blight. Rept. Colo. Agr. Exp. Sta. 1: 64-70.
1898. Waite, M. B. Fungous diseases of the apple and pear. Proc. Mich. Hort. Soc. 1897: 186-191.
1902. Isaac, John. Pear blight in California. Rept. Cal. State Board Hort. 8: 53-65.
1902. Jones, L. R. Studies on plum blight. Rept. Vermont Agr. Exp. Sta. 15: 231-239.

1902. Pierce, Newton B. Pear blight in California. Science,
n. s. 16: 193-194.
1903. Paddock, Wendall. An apricot blight. Colo. Agr. Exp. Sta.
Bul. 84: 5-14.
1904. Harrison, F. C. Some bacterial diseases of plants prevalent
in Ontario. Fire blight or twig blight. Ontario
Agr. Col. Bul. 136: 1-9.
1905. Sackett, W. G. Bacterial diseases in plants. Pear blight.
Mich. Agr. Exp. Sta. Bul. 230: 205-210.
1905. Smith, E. F. Bacteria in relation to plant diseases. 1: 1-285.
1906. Smith, R. E. Pear blight. Ann. Rept. Cal. Fruit Growers
Assoc. 31: 137-161.
1906. Waite, M. B., and Smith, R. E. Pear blight. Ann. Rept. Cal.
Fruit Growers Assoc. 31: 137-161.
1906. Whetzel, H. H. The blight canker of apple trees. N. Y.
(Cornell) Agr. Exp. Sta. Bul. 236: 104-138.
1906. Whetzel, H. H. Fire blight and apple tree canker. Ann. Rept.
Wis. Hort. Soc. 36: 215-226.
1907. Lawrence, W. H. Important diseases of Washington. Fire blight.
Wash. Agr. Exp. Sta. Bul. 83: 19+21.
1907. Waite, M. B. A new native host for pear blight. Science, n. s.
25: 286-287.
1909. Jones, D. H. Bacterial blight of apple, pear, and quince trees.
Ontario Agr. Col. Bul. 176: 1-63.
1909. Sackett, W. G. Some bacterial diseases of plants. Pear blight.
Colo. Agr. Exp. Sta. Bul. 138: 6-14.
1909. Smith, R. E. Report of plant pathologist. Pear blight. Cal.
Agr. Exp. Sta. Bul. 203: 18-24.

1911. Fulton, H. R. The persistence of Bacillus amylovorus in pruned apple twigs. *Phytopath.* 1: 68.
1911. Sackett, W. G. Hold-over blight in the pear. *Colo. Agr. Exp. Sta. Bul.* 177: 2-8.
1911. Smith, E. F. Bacteria in relation to plant diseases. 2: 1-368.
1911. Smith, E. F., Brown, Nellie A., and Townsend, C. O. Crown gall of plants: its cause and remedy. Crown gall followed by hold-over blight. U. S. Dept. Agr., Bur. Pl. Indus. *Bul.* 213: 186.
1911. Smith, R. E. and Smith, Elizabeth H. California plant diseases. Pear blight. *Cal. Agr. Exp. Sta. Bul.* 218:1154-1155.
1911. Swingle, Deane B. Pear and apple blight in Montana. *Mont. Agr. Exp. Sta. Cir.* 2: 1-14.
1913. Hall, John G. Fire blight of pear and apple. *Wash. Agr. Exp. Sta. Pop. Bul.* 56: 1-8.
1913. Jackson, H. S. Fire blight of pear and apple. *Oregon Agr. Exp. Sta. Cir. Bul.* 7: 1-15.
1913. Stewart, V. B. The fire blight disease in nursery stock. N. Y. (Cornell) *Agr. Exp. Sta. Bul.* 329: 314-371.
1914. Pickett, B. S. The blight of apples, pears, and quinces. *Ill. Agr. Exp. Sta. Cir.* 172: 1-10.
1915. Jackson, H. S. Notes and observations on plant diseases. Fire blight on prune. *Biennial Crop Pest and Hort. Rept. Oregon Agr. Exp. Sta.* 2: 279-281.

Plate I

Diseased and healthy seedlings

Plate I



Plate 2

**Twig blight showing healthy shoot
below diseased area. Collected fall of
1914.**

Plate 2



Plate 3

Large limb cankers

Plate 3



Plate 4

Body cankers.

**(Infection taking place through
tender shoot).**

Plate 4



Approved

L. R. Jones

[illegible]

Date Due

[illegible]

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